Cherish your doubts, for doubt
is the attendant of the truth

Part A

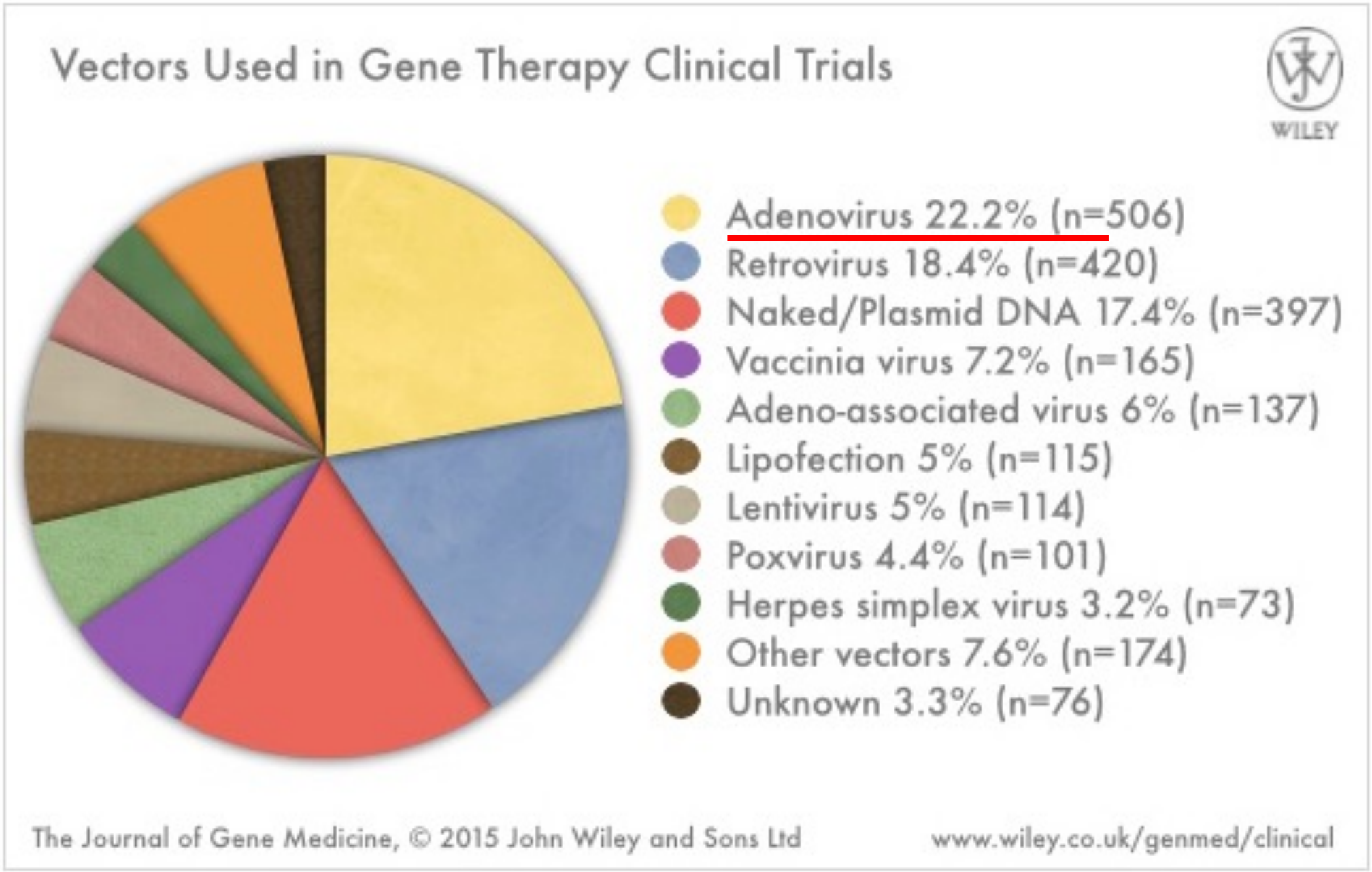
Future - infos

Open desk for infos thesis and future

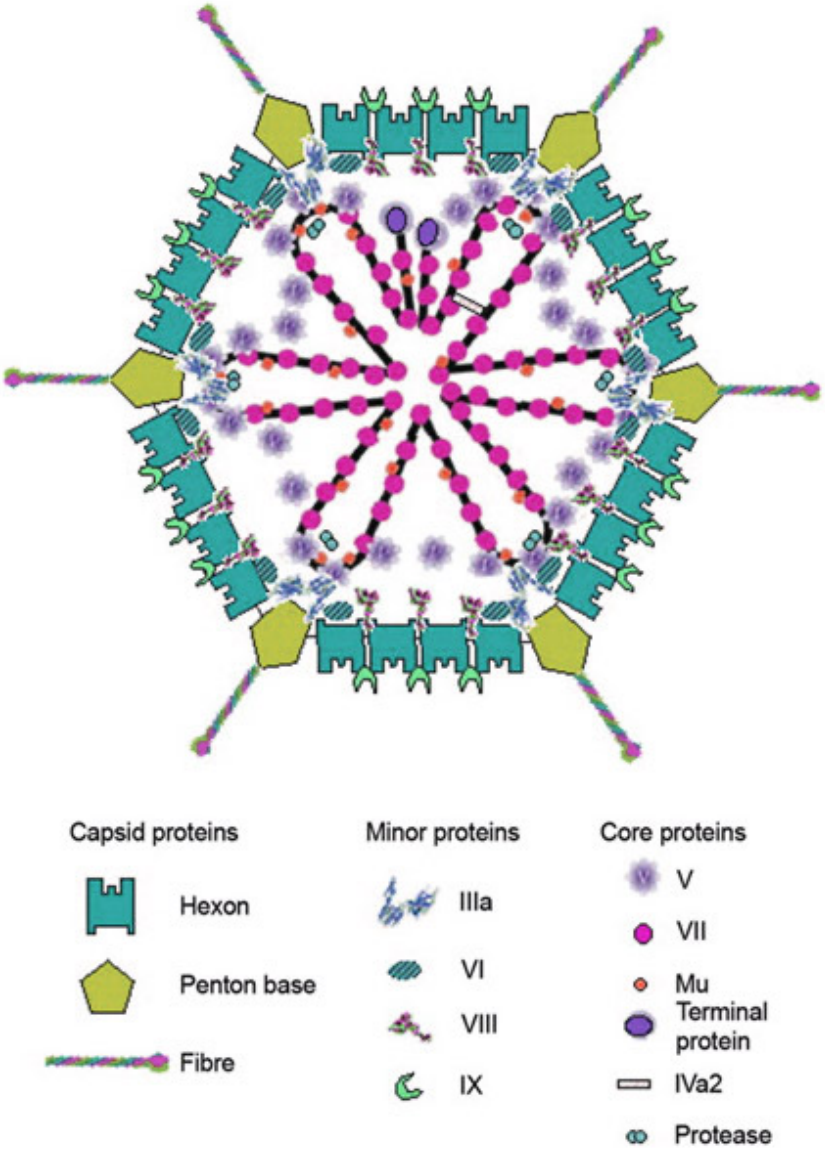
Long term future:

Master sgp
Master One Health analyst

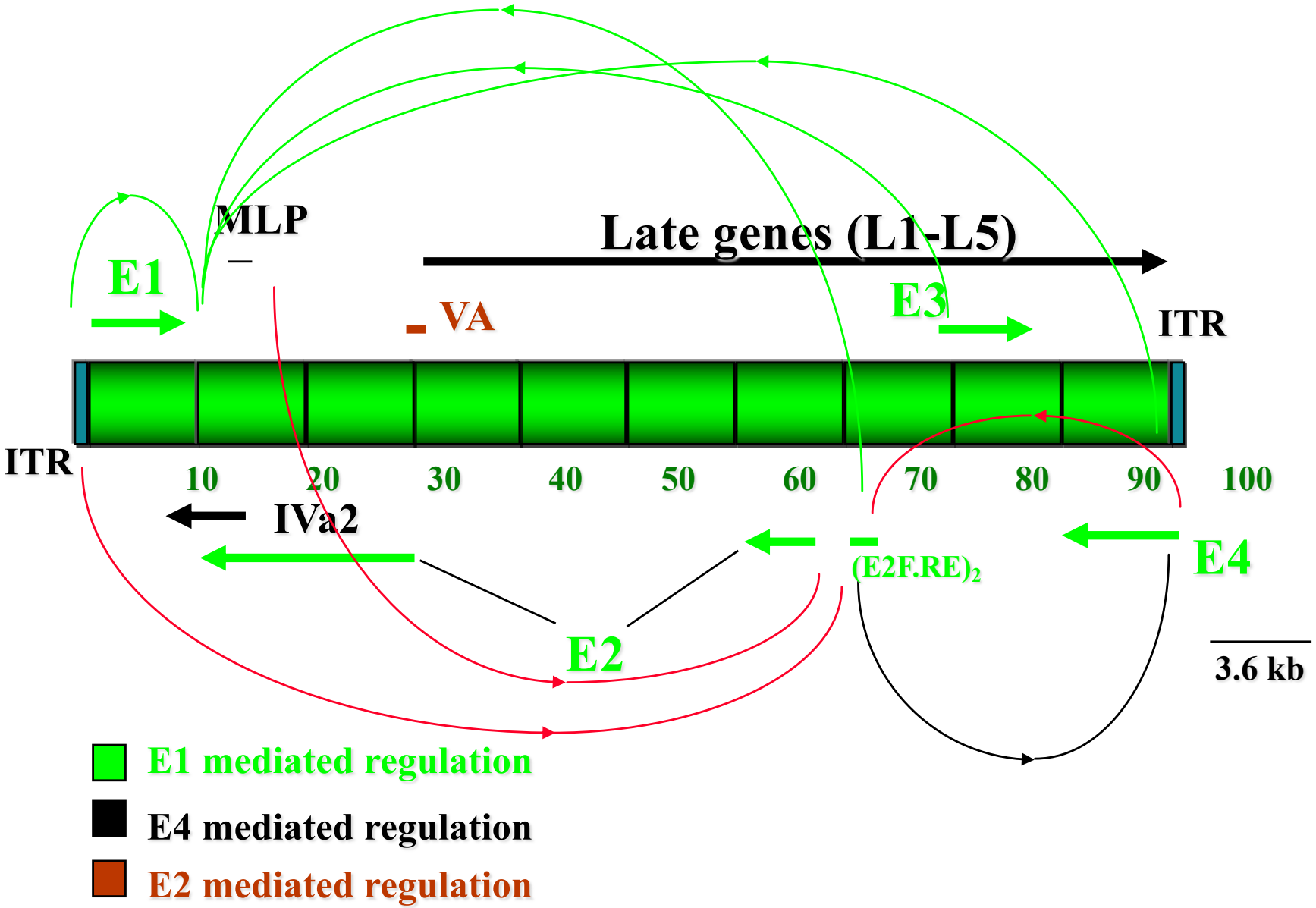
Top down


Vectors Used in Gene Therapy Clinical Trials
WILEY
Adenovirus 22.2% (n=506)
Retrovirus 18.4% (n=420)
Naked/Plasmid DNA 17.4% (n=397)
Vaccinia virus 7.2% (n=165)
Adeno-associated virus 6% (n=137)
Lipofection 5% (n=115)
Lentivirus 5% (n=114)
Poxvirus 4.4% (n=101)
Herpes simplex virus 3.2% (n=73)
Other vectors 7.6% (n=174)
Unknown 3.3% (n=76)
The Journal of Gene Medicine, © 2015 John Wiley and Sons Ltd
www.wiley.co.uk/genmed/clinical

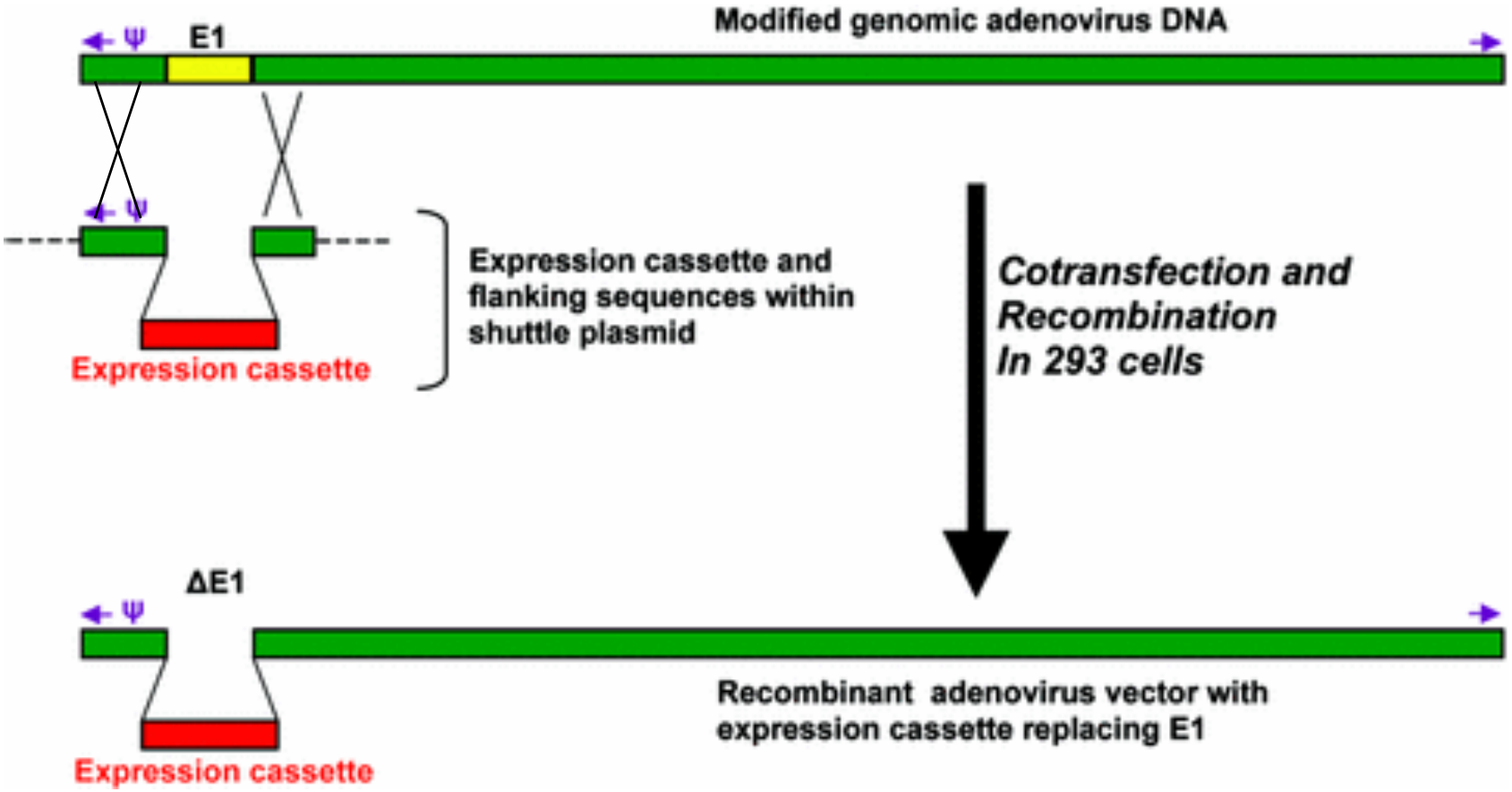
Adenovirus


Capsid proteins
Hexon
Penton base
Fibre
Minor proteins
IIIa
VI
VIII
IX
Core proteins
V
VII
Mu
Terminal protein
IVa2
Protease

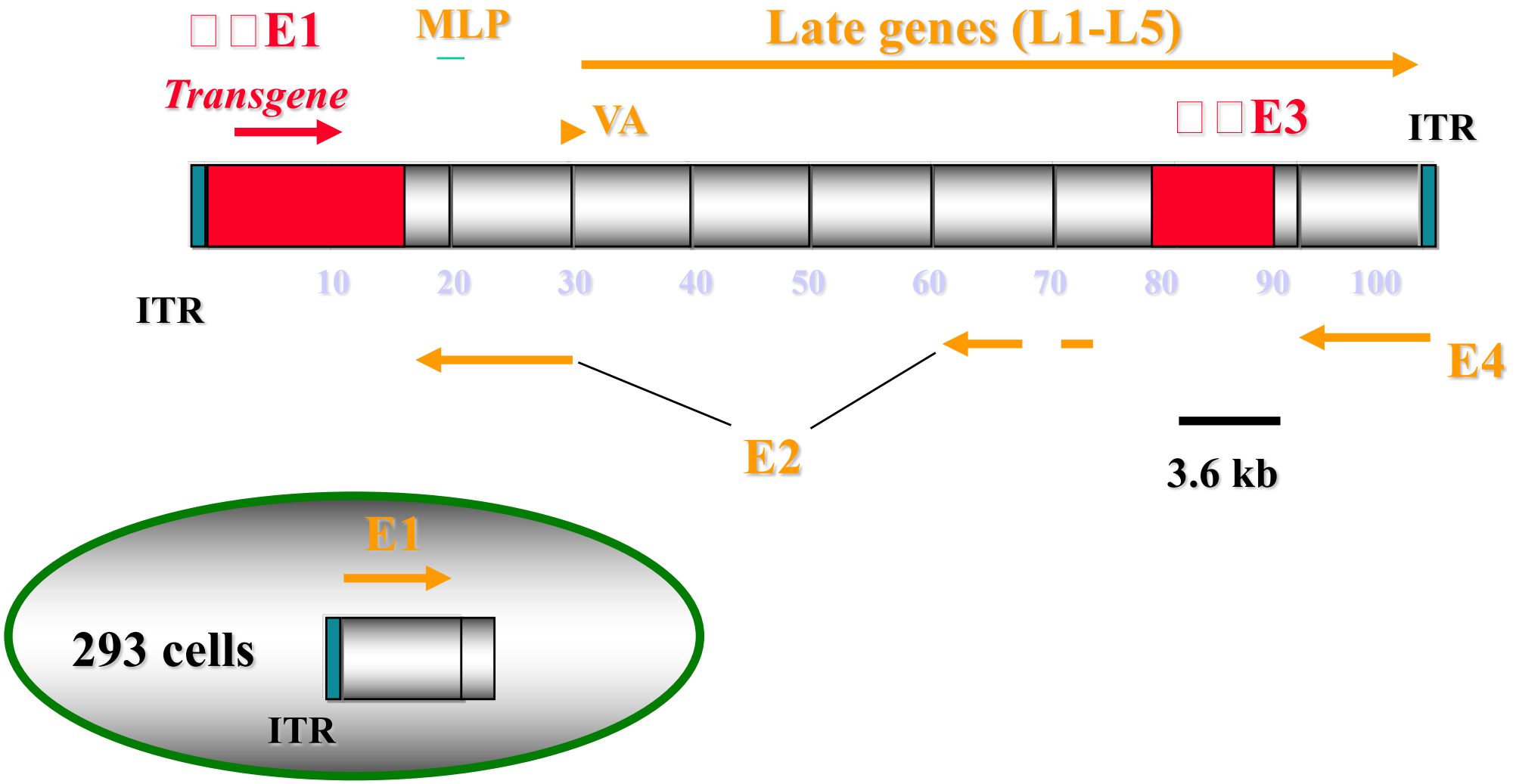
Adenovirus genome



1st generation adenoviral vectors



1st generation adenoviral vectors

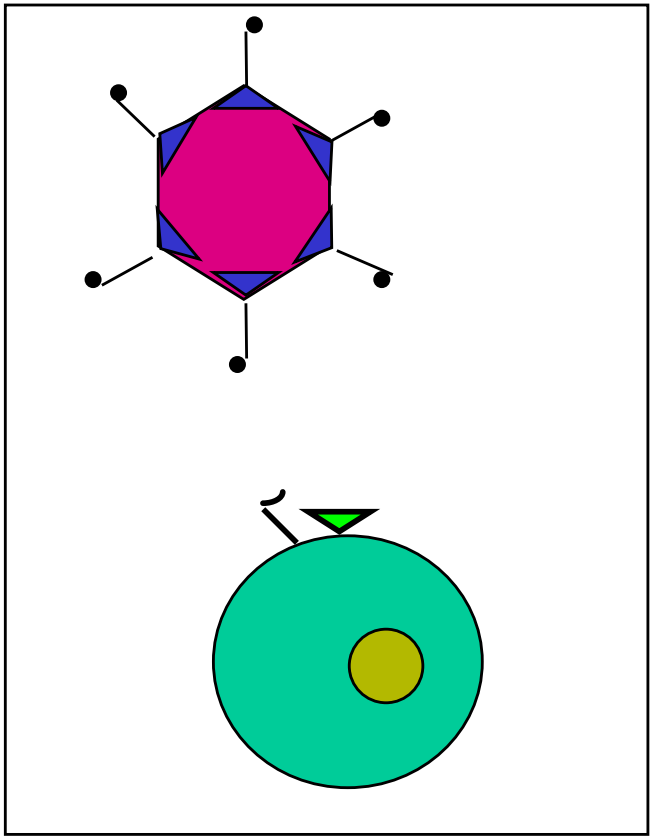


Problems and ameliorations of Ad vectors

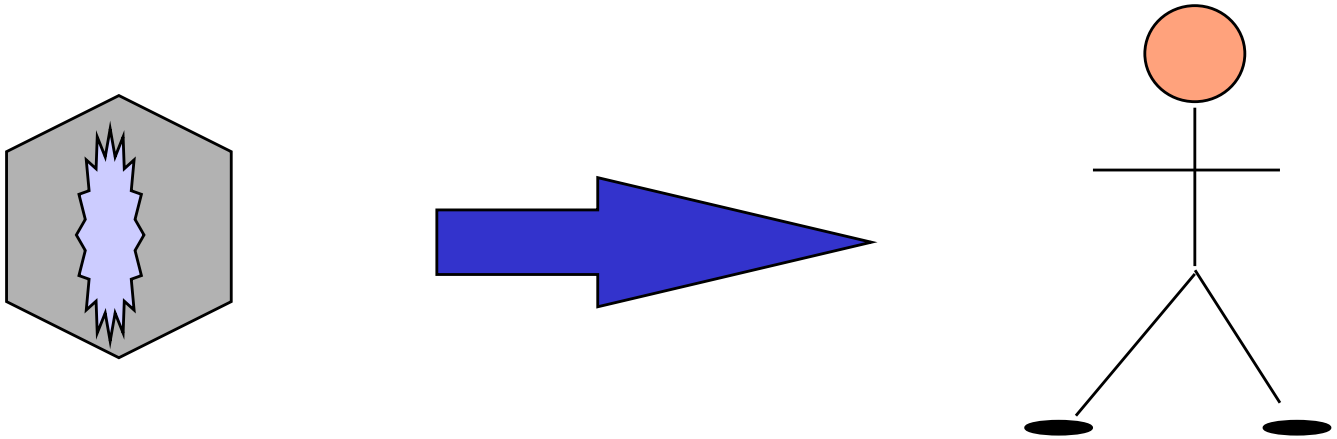
- no integration => chimaeres AAV/ Retro
- seropositivity to Ad => change of serotype, higher doses, immunosuppression
- large tropism => **targeted transduction**, targeted expression
- immunogenicity => **immuno-suppression**, **new vectors**
- size of the insert => new vectors
- short term expr. => chimaeres AAV/Retro, immuno-suppression, new generation vectors
- RCA => new lines, new vectors
- transcomplementation => new vectors

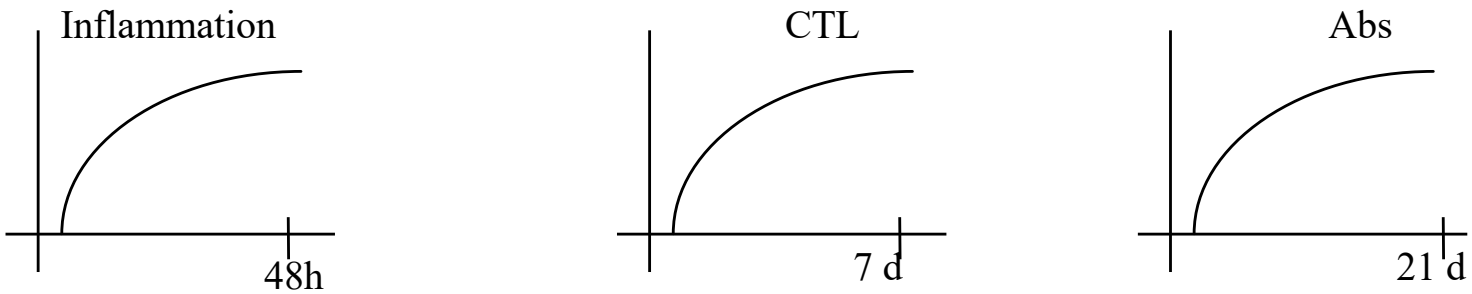
Ad modifications for targeting

- bispecific ABs antifiber/antireceptor (nabs)
- bispecific abs anti fiber insert/antireceptor (antiflag/antireceptor)
- fiber inserts (RGD)
- hexon inserts
- penton base inserts

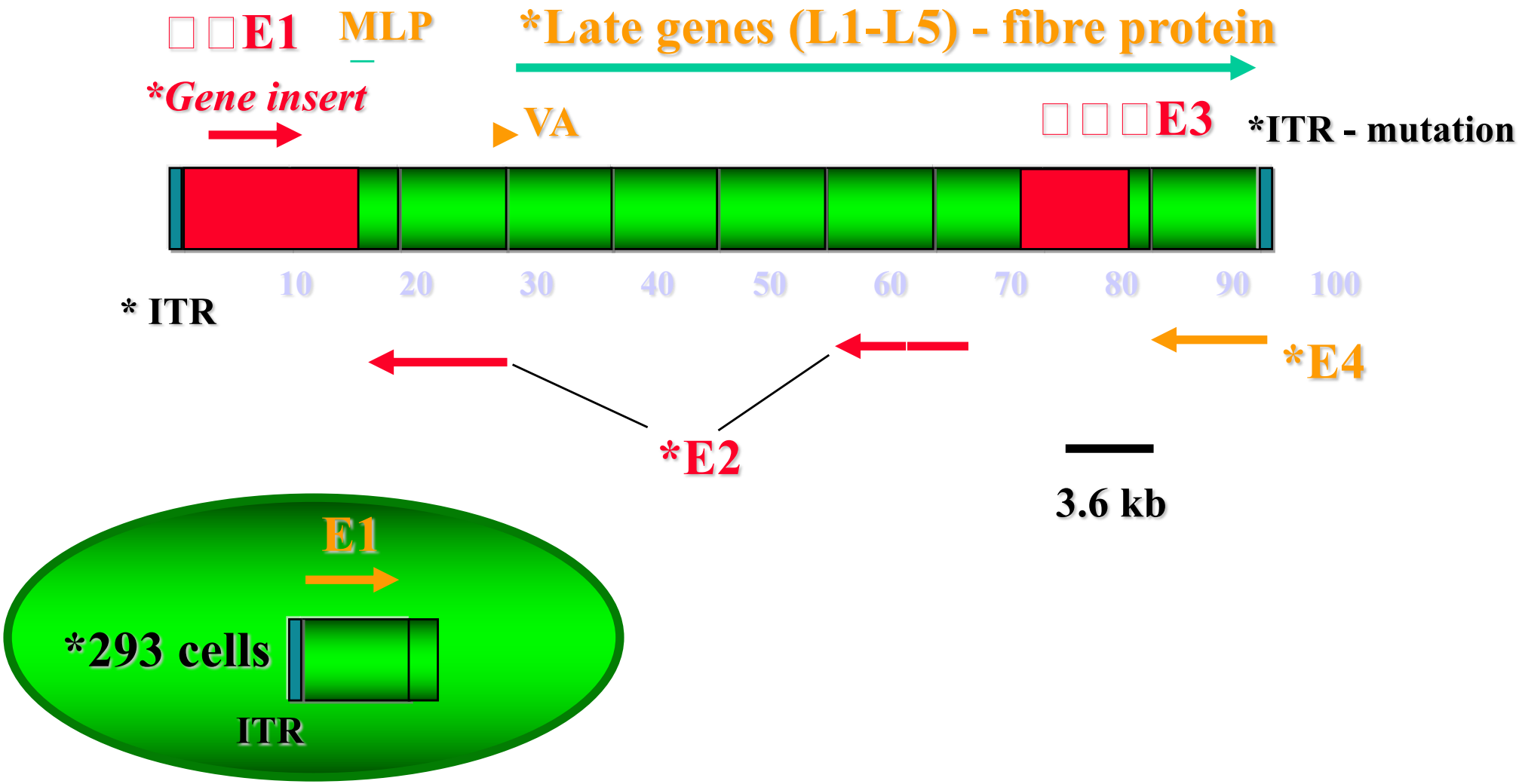


Immune response to adenoviral vectors

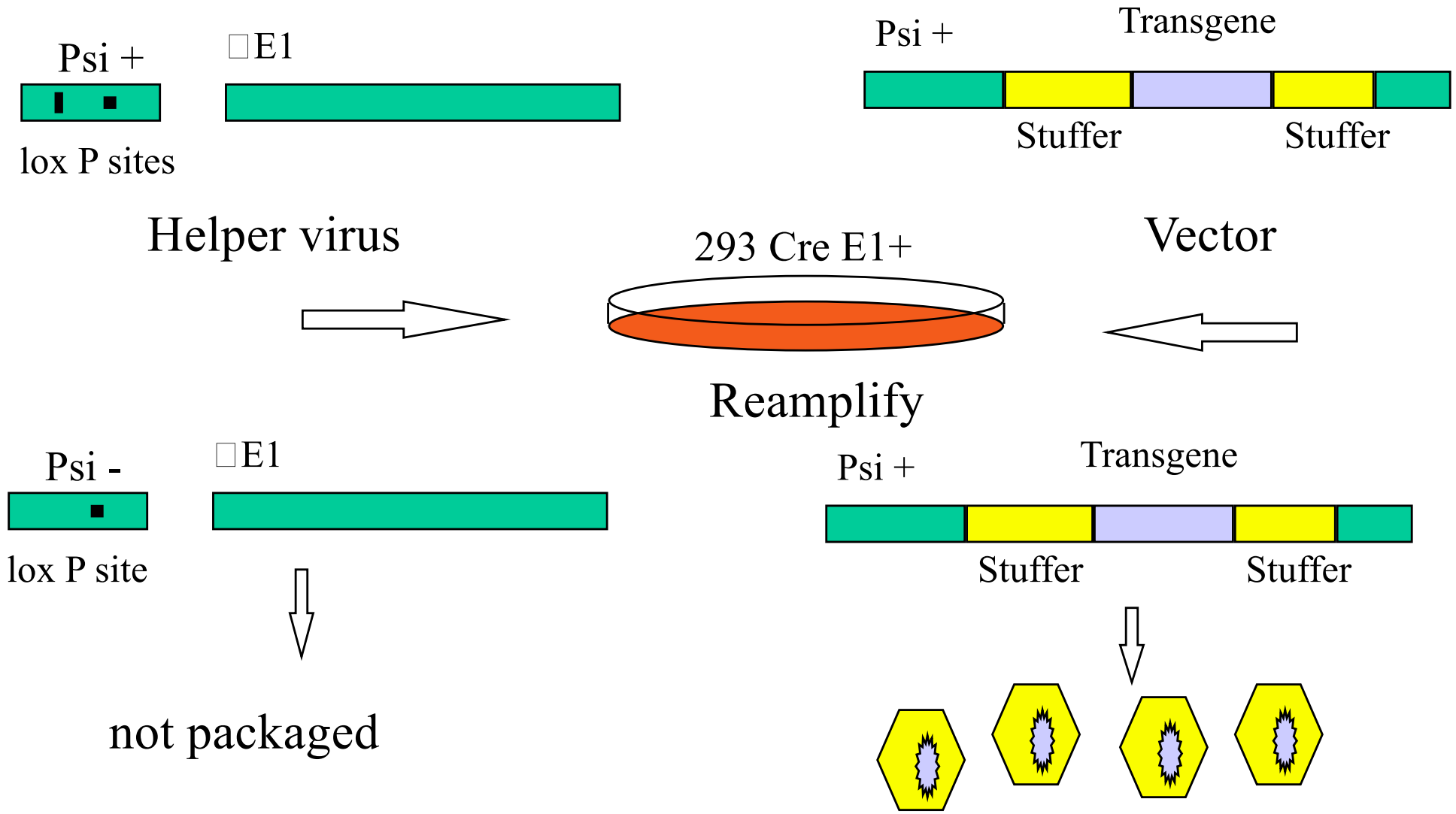




2nd generation Adenoviral vectors



3rd generation Adenoviral vectors



3rd generation Ad vectors: advantages

- size of the insert (36kb)
- low immunogenicity (no viral sequences)
- long term expression

3rd generation ad vectors: disadvantages

- titers
- instability
- helper contaminations
- stuffer?

Part B

Adenovirus mediated gene therapy: history

- *Welsh Cell 1993* Adenovirus mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with cystic fibrosis
- *Wilson Nature Genetics1993* Gene therapy in a xenograft model of cystic fibrosis lung corrects chloride transport more effectively than the sodium defect
- *Peschanski Nature Genetics 1993* Transfer of a foreign gene into the brain using adenovirus vectors
- *Wilson 1993* Direct gene transfer of human CFTR into human bronchial epithelia of xenografts with E1-deleted adenoviruses

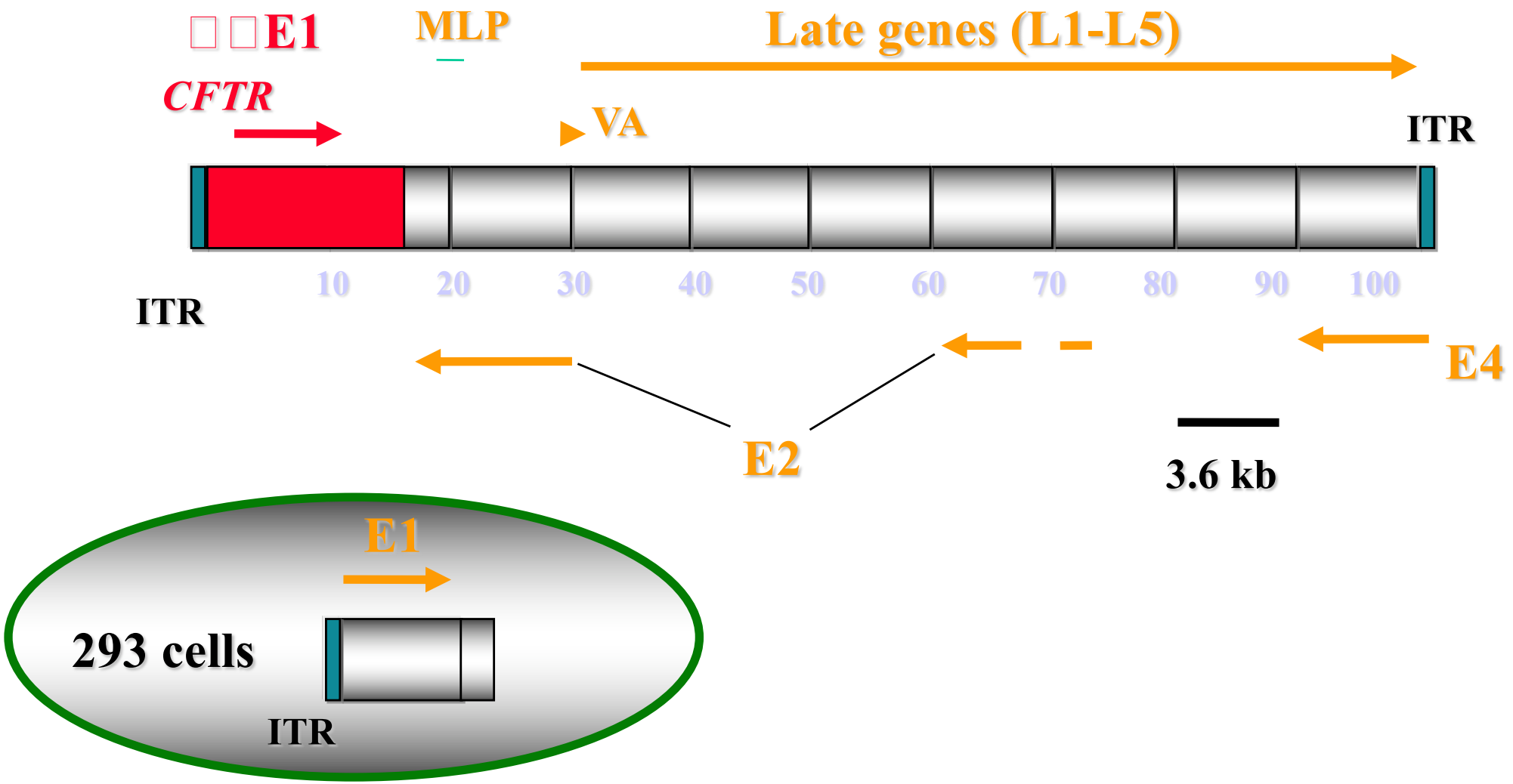
Ad-mediated gene therapy: history (follows)

- *Crystal Nature Genetics 1994* Administration of an adenovirus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis
- *Wilson Nature Genetics 1996* Effective treatment of familial hypercolesterolaemia in the mouse model using adenovirus-mediated transfer of the VLDL receptor gene
- *McCormick Science 1996* An adenovirus mutant that replicates selectively in p53 deficient human tumor cells

Cystic fibrosis gene therapy

- Autosomal recessive disease caused by mutations in the transmembrane conductance regulator (CFTR)
- The Cl- channel is deregulated => defective Cl- transport => lung disease

1st generation Ad-CFTR



Cell 93: AdCFTR in human patients

Check of ion transport:

amiloride creates a gradient and if the channel works, terbutaline makes Cl^- going out

Healthy patient

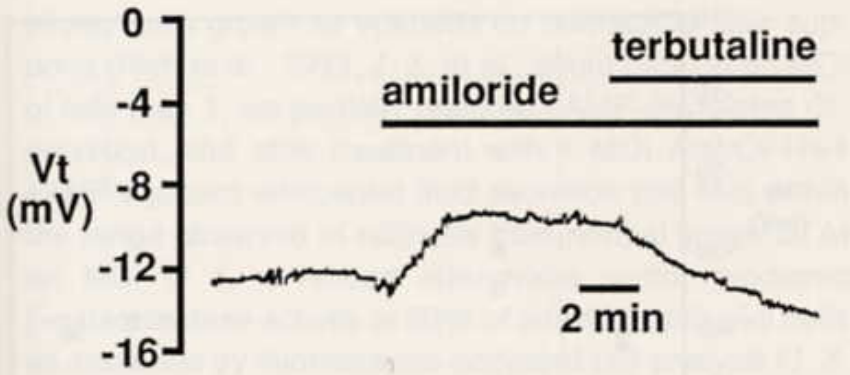
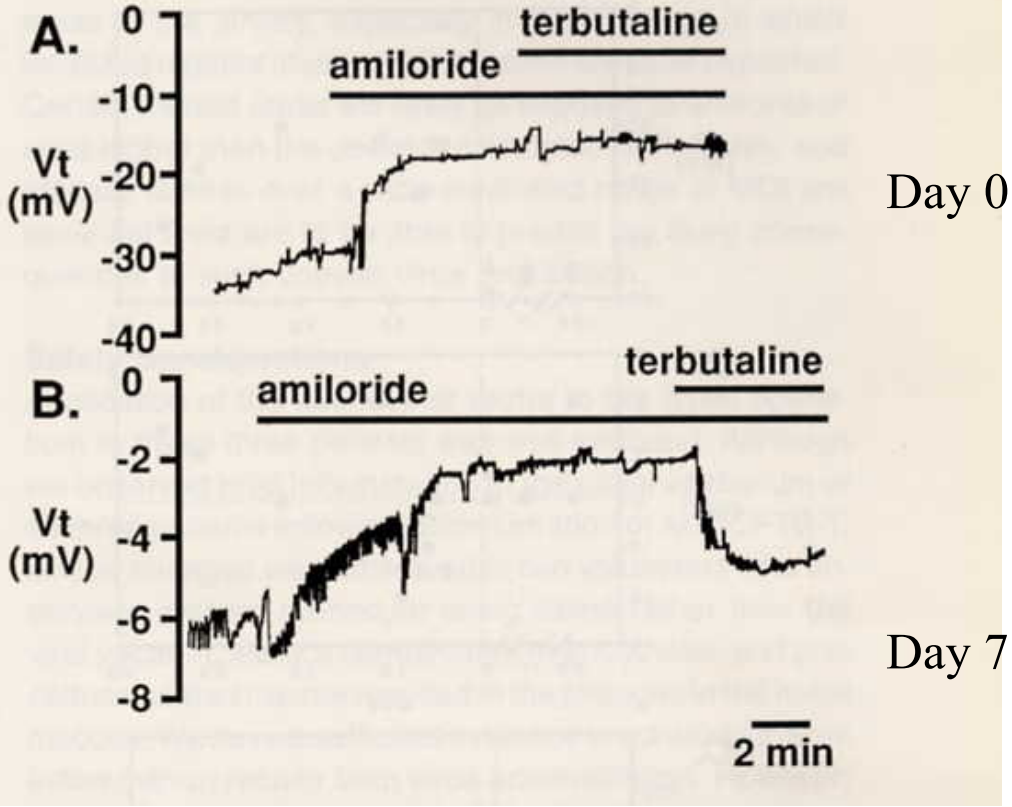


Figure 3. Measurement of Vt across the Nasal Epithelium of a Normal Subject

Amiloride (100 μM) and terbutaline (10 μM) were perfused onto the mucosal surface during times indicated by the bars.

fect cannot be attributed to the anesthesia/application procedure because it did not occur in patients treated with saline instead of Ad2/CFTR-1 (Figure 7). Moreover, the effects of the anesthesia were generalized on the nasal

CF patient +AdCFTR



Day 0

Day 7

Ad-CFTR

Nature Genetics 93/94

"*Semi-in vivo*"
AdCFTR in human
bronchial xenografts

Bronchus ->

Xenograft ->

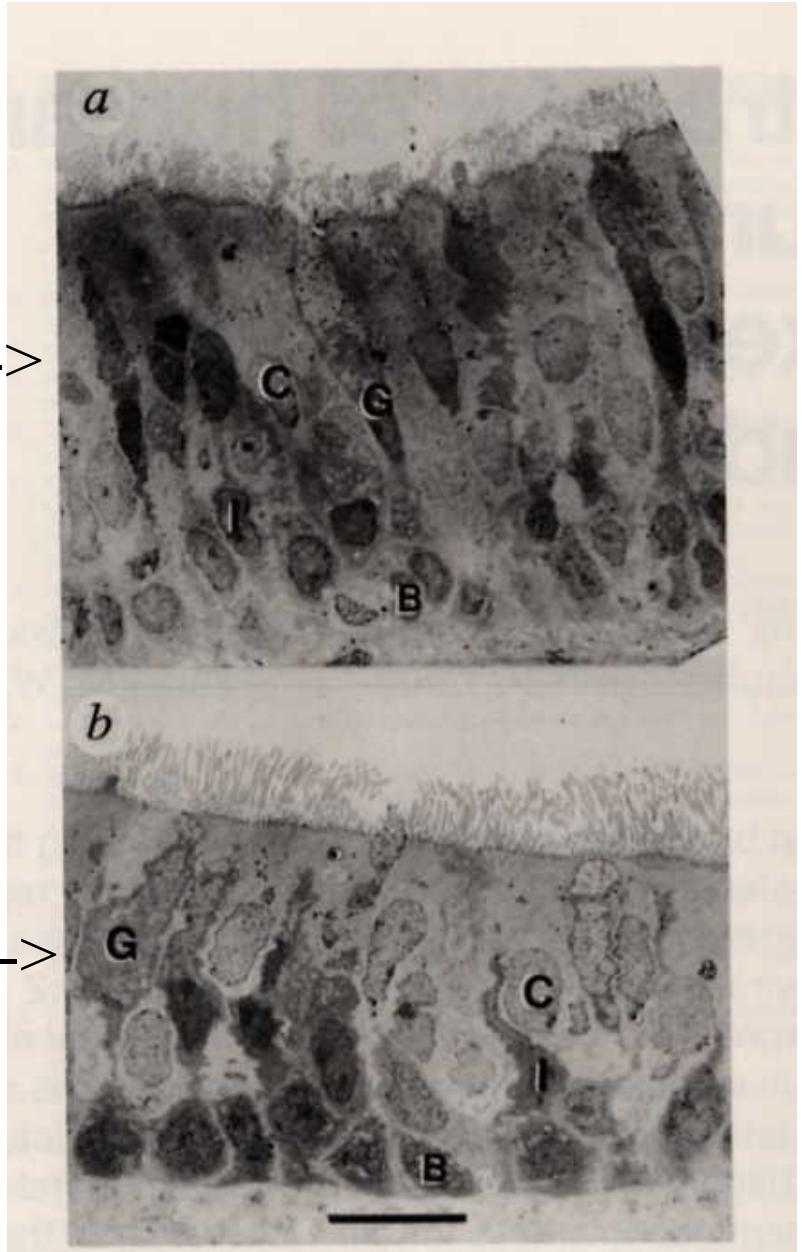
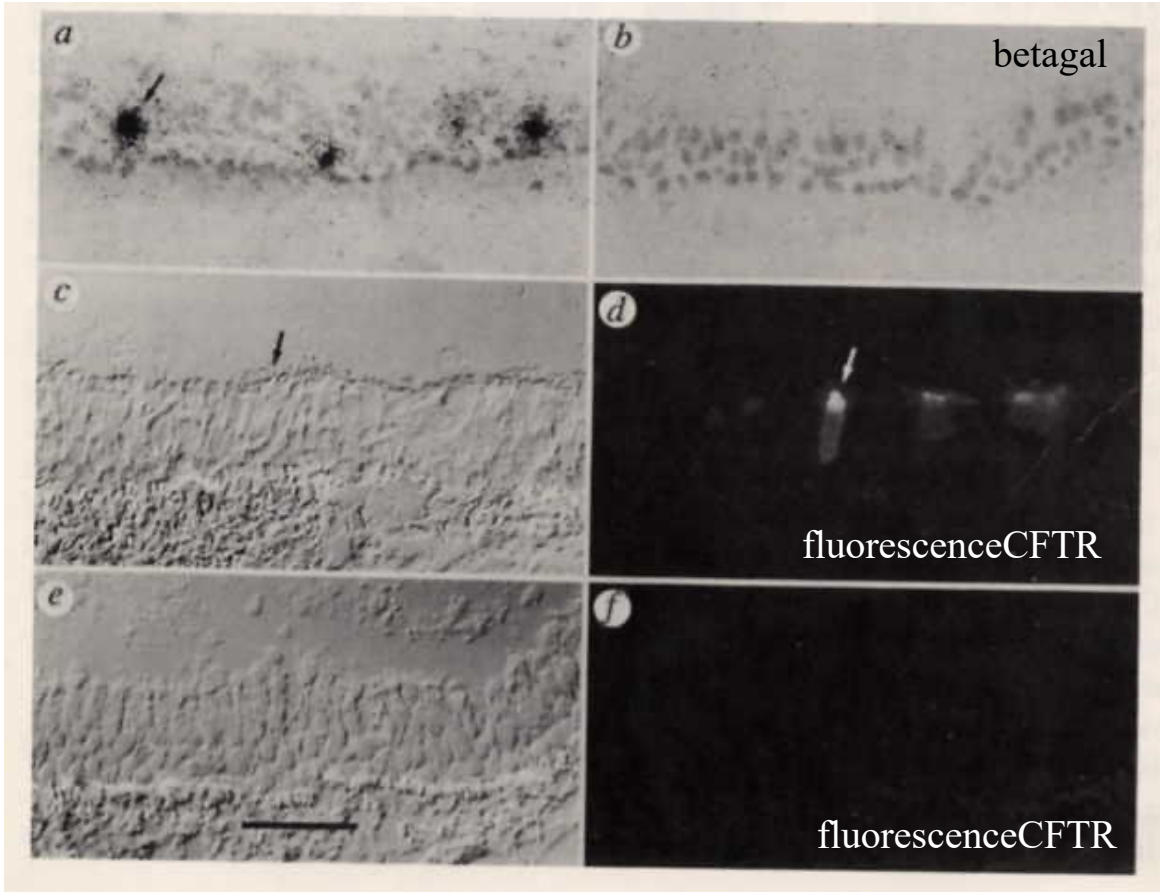


Fig. 1 Electron micrographs of bronchial epithelia from human bronchus and a xenograft. Micrograph of human bronchial epithelium *a*, and epithelium from a xenograft seeded with human bronchial epithelial cells and harvested at 42 days *b*. C ciliated cell; G, goblet cell; B, basal cell and I, intermediate cell

AdCFTR in human bronchial epithelial xenografts; 1 week after infection



Adbgal

AdCFTR

Adbgal

Nat Gen 93 Wilson/Perricaudet

Ion function in AdCFTR infected human bronchial xenografts

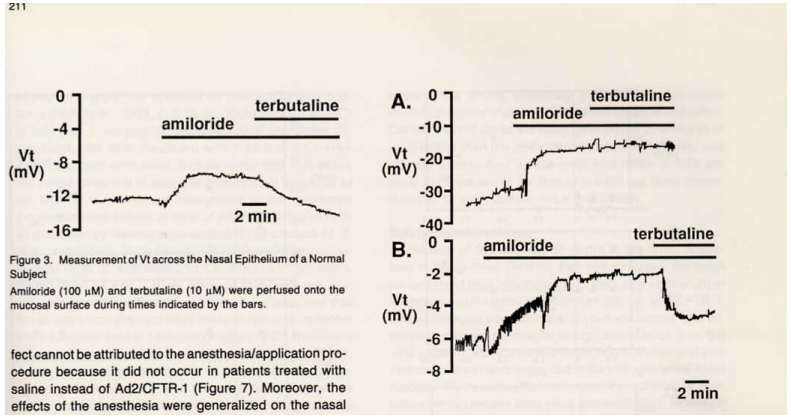


Figure 3. Measurement of Vt across the Nasal Epithelium of a Normal Subject

Amiloride (100 μM) and terbutaline (10 μM) were perfused onto the mucosal surface during times indicated by the bars.

fect cannot be attributed to the anesthesia/application procedure because it did not occur in patients treated with saline instead of Ad2/CFTR-1 (Figure 7). Moreover, the effects of the anesthesia were generalized on the nasal

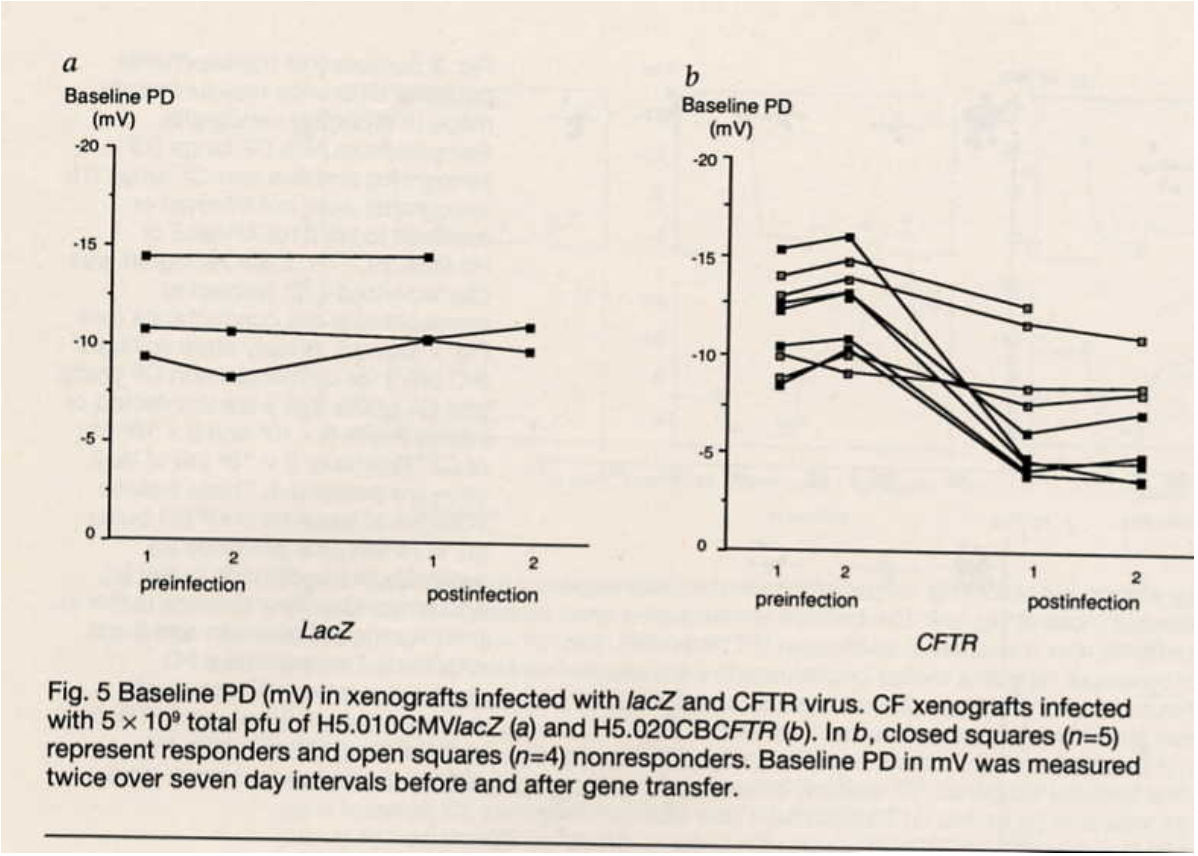


Fig. 5 Baseline PD (mV) in xenografts infected with *lacZ* and CFTR virus. CF xenografts infected with 5×10^9 total pfu of H5.010CMV*lacZ* (*a*) and H5.020CB*CFTR* (*b*). In *b*, closed squares (n=5) represent responders and open squares (n=4) nonresponders. Baseline PD in mV was measured twice over seven day intervals before and after gene transfer.

Nat Gen 93 Wilson/Perricaudet

Ad-residual activity in bronchial xenografts

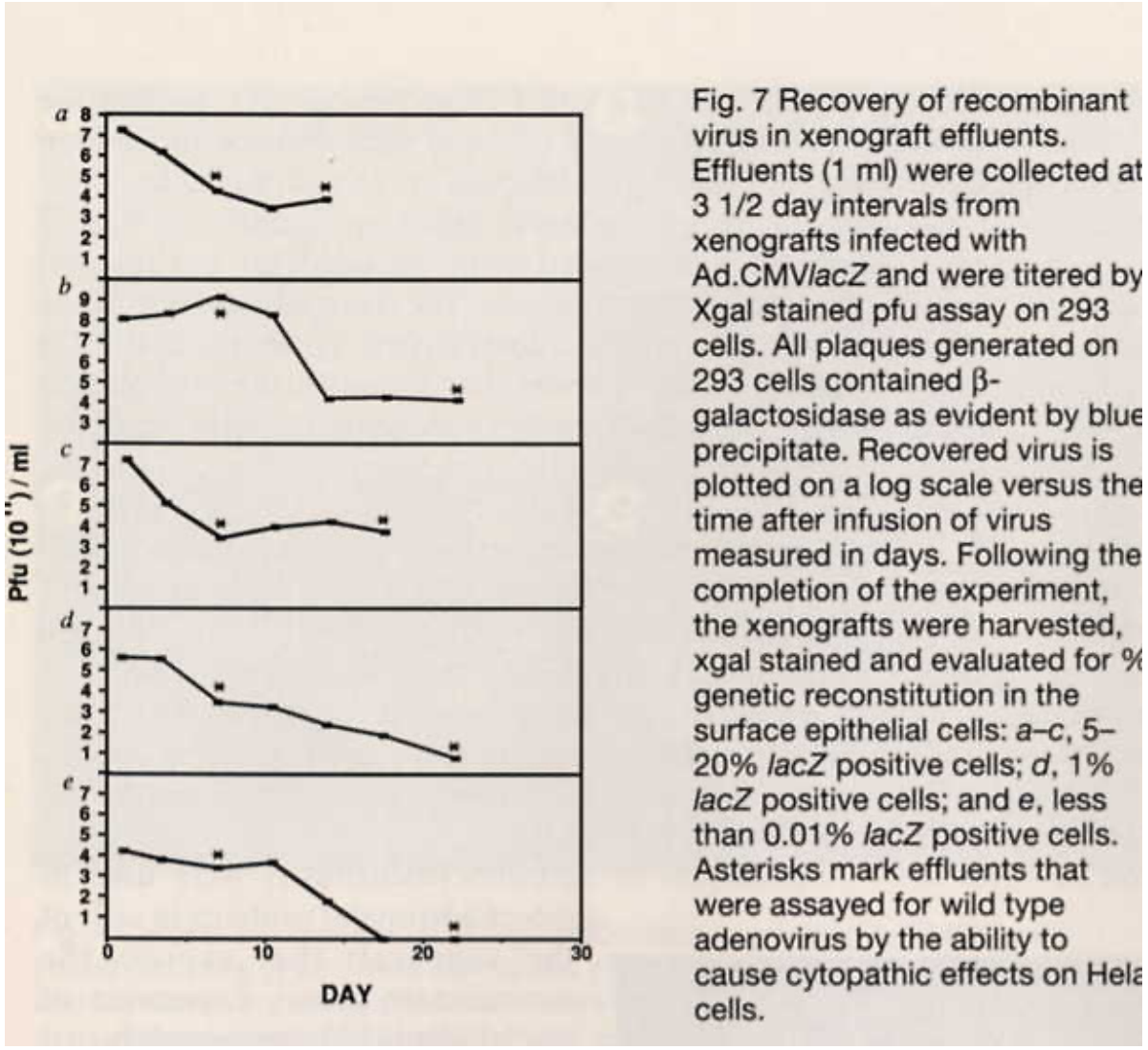


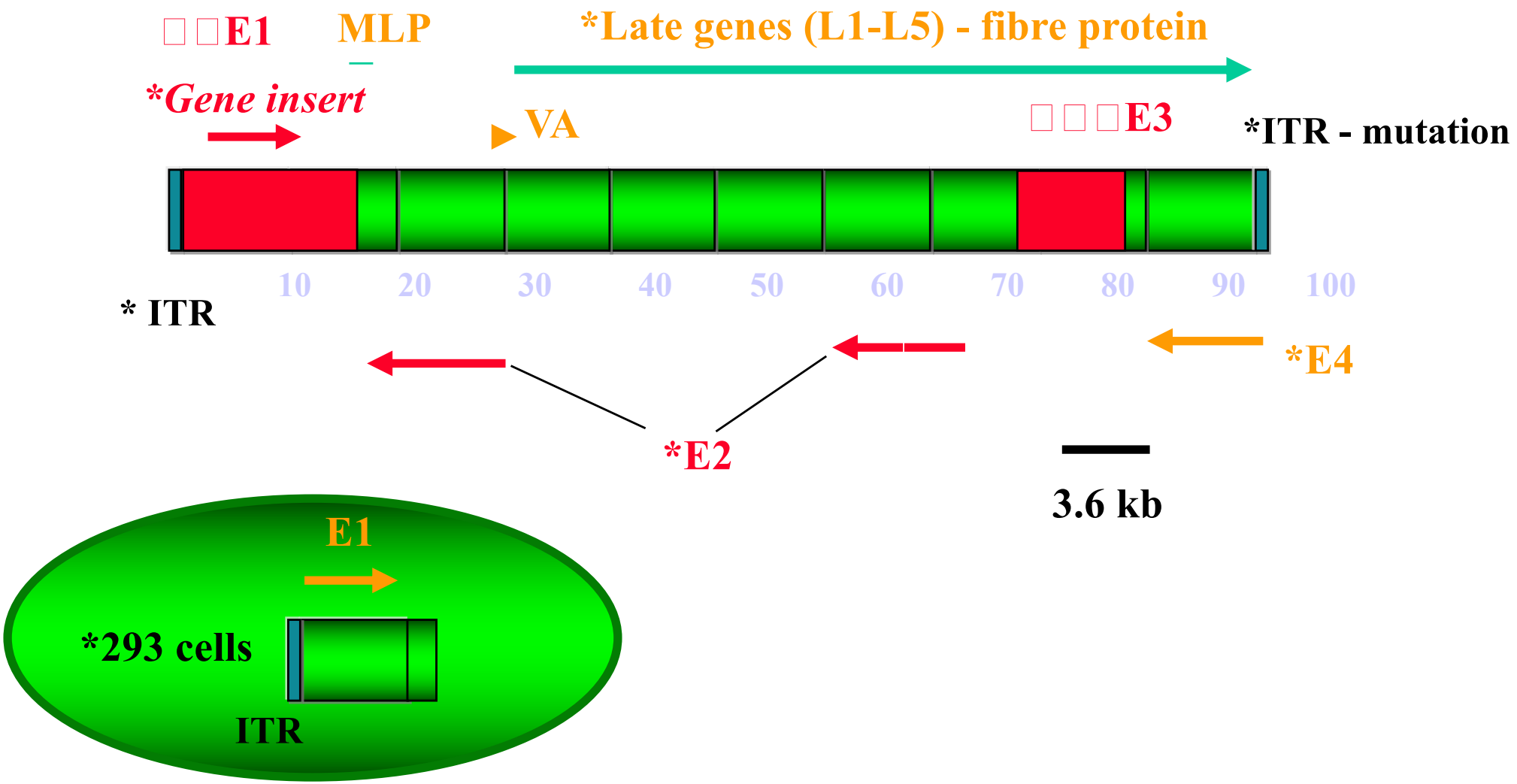
Fig. 7 Recovery of recombinant virus in xenograft effluents. Effluents (1 ml) were collected at 3 1/2 day intervals from xenografts infected with Ad.CMV*lacZ* and were titered by Xgal stained pfu assay on 293 cells. All plaques generated on 293 cells contained β-galactosidase as evident by blue precipitate. Recovered virus is plotted on a log scale versus the time after infusion of virus measured in days. Following the completion of the experiment, the xenografts were harvested, xgal stained and evaluated for % genetic reconstitution in the surface epithelial cells: *a–c*, 5–20% *lacZ* positive cells; *d*, 1% *lacZ* positive cells; and *e*, less than 0.01% *lacZ* positive cells. Asterisks mark effluents that were assayed for wild type adenovirus by the ability to cause cytopathic effects on Hela cells.

Nat Gen 93 Wilson/Perricaudet

Problems and ameliorations of Ad vectors

- no integration => chimaeres AAV/ Retro
- seropositivity to Ad => change of serotype, higher doses, immunosuppression
- large tropism => **targeted transduction**, targeted expression
- immunogenicity => **immuno-suppression**, **new vectors**
- size of the insert => new vectors
- short term expr. => chimaeres AAV/Retro, immuno-suppression, new generation vectors
- RCA => new lines, new vectors
- transcomplementation => new vectors

2nd generation adenoviral vectors



Adeno-death
(clinical trials Wilson)

- 18 year old boy
- To correct ornithyne transcarbamylase deficiency (OCT), a metabolic disease that can induce ammonia accumulation in the body
- Ad-OCT 3.8 x10e13 2nd generation vector (E1-deleted, E2A-temperature-sensitive) in the hepatic artery
- Patient dyes 4 days after injection

Why?

CLINICAL TRIALS

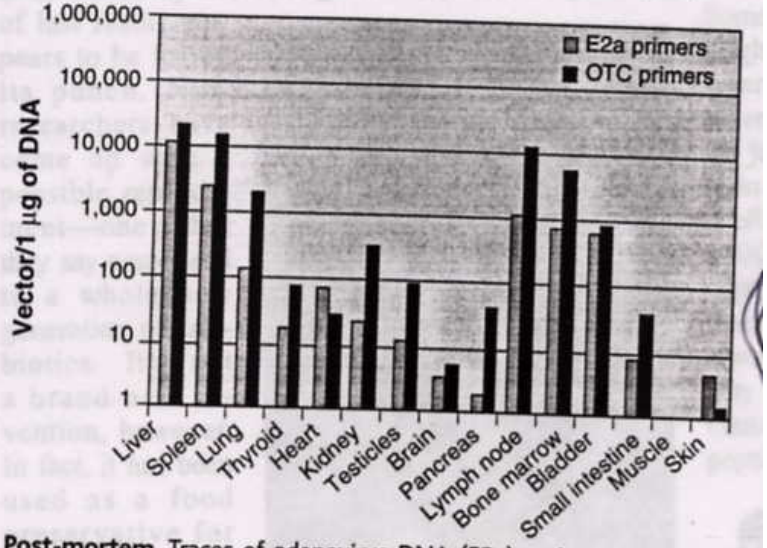
Gene Therapy Death Prompts Review of Adenovirus Vector

For the past 3 months, one-third of the 250 faculty and staff members connected with the University of Pennsylvania's Institute for Human Gene Therapy have been studying a single case. They've been trying to understand why Jesse Gelsinger, a relatively fit 18-year-old with an inherited enzyme deficiency, died on 17 September, 4 days after doctors at Penn injected a genetically altered virus into his liver.

Gelsinger was the first patient in a gene therapy trial to die of the therapy itself, as James Wilson, who heads the Penn institute, confirmed at a public meeting last week. His death is the latest blow to a field that has been struggling to live up to the promise and hype surrounding the first gene therapy trials a decade ago. And Penn isn't the only one investigat-

Gelsinger had died. It was a tense session.

After releasing stacks of clinical data and answering questions for 2 days, however, Wilson and colleagues said that they didn't fully understand what had gone amiss. They report-



Post-mortem. Traces of adenovirus DNA (E2a) and a curative gene (OTC) it carried turned up in many tissues outside the patient's target organ, the liver.

Science 1999 Marschall

Sanctions agreed over teenager's gene therapy death

- 5 year investigation
- According to an investigation by the university, Gelsinger died from an immune reaction to the adenovirus vector.
- The justice department alleged that the researchers and their institutions made false statements regarding the safety of the trial to the National Institutes of Health, the Food and Drug Administration, and the institutional review board that oversaw the research.
- 3 researchers will pay 1 million $
- The terms of the settlement state that a monitor will supervise Wilson's work in humans for three years, and he will be allowe to conduct only one trial at a time. Any of Wilson's animal research that could affect patient safety will also be supervised
- Wilson : retraining for clinical trial, clinical trials in 2010

Nature, 2005

http://www.nih.gov/catalyst/2000/00.01.01/page1.html

NEWS · 19 OCTOBER 2017

FDA advisers back gene therapy for rare form of blindness

Therapy that targets disease-causing mutations could become the first of its kind approved for use in the United States.

But some researchers kept plugging away at the problem, improving the vectors that shuttle genes into human cells. Over time, new clinical trials began to show promise, and pharmaceutical companies became more interested in developing treatments for rare genetic diseases. Gradually, investors returned.

Now, demand for gene-therapy vectors is so high that suppliers are oversubscribed, and researchers have to wait between 18 months and 2 years to get some of the reagents that they need for clinical studies, says Williams.

On 12 October, a panel of external experts unanimously voted that the benefits of the therapy, which treats a form of hereditary blindness, outweigh its risks. The FDA is not required to follow the guidance of its advisers, but it often does. A final decision on the treatment, called voretigene neparvovec (Luxturna), is expected by 12 January.

> An approval in the lucrative US drug market would be a validation that gene-therapy researchers have awaited for decades. “It’s the first of its kind,” says geneticist Mark Kay of Stanford University in California, of the treatment. “Things are beginning to look more promising for gene therapy.”

Gene replacement

Luxturna is made by Spark Therapeutics of Philadelphia, Pennsylvania, and is designed to treat individuals who have two mutated copies of a gene called RPE65. The mutations impair the eye’s ability to respond to light, and ultimately lead to the destruction of photoreceptors in the retina.

In a randomized controlled trial that enrolled 31 people, Spark showed that, on average, patients who received the treatment improved their ability to navigate a special obstacle course. This improvement was sustained for the full year during which the company gathered data. The control group, however, showed no improvement overall. This was enough to convince the FDA advisory committee that the benefits of the therapy outweigh the risks.

Long road

That endorsement is an important vote of confidence for a field that has struggled over the past 20 years. In the early 1990s, gene therapy was red hot, says David Williams, chief scientific officer at Boston Children's Hospital in Massachusetts. "You couldn't keep young people out of the field," he says. "Everyone wanted in." Then came the death of a young patient enrolled in a gene-therapy clinical trial, and the realization that a gene therapy used to treat children with an immune disorder could cause leukaemia.

Investors backed away from gene therapy, and some academics grew scornful of it. Although European regulators approved one such therapy in 2012, for a condition that causes severe pancreatitis, many doubted that it worked. (The company that makes it has announced that it will not renew its licence to market the drug when it expires on 25 October.) "You're too smart to work in this field," a colleague told Kay. "It's a pseudoscience."